Transcriptional control: **Tat cofactors and transcriptional elongation**

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HIV-1 gene expression requires the transactivator Tat, which stimulates viral transcript elongation. Recent results show that two cellular cyclin-dependent kinases, which phosphorylate the carboxy-terminal domain of the RNA polymerase II large subunit, contact Tat and contribute to the control of transcriptional elongation.

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Current Biology 1998, 8:R447–R449
http://biomednet.com/elecref/09609822008R0447

The transcriptional activator protein Tat of human immundeficiency virus-1 (HIV-1) enhances synthesis of viral RNA by a very unusual mechanism. Firstly, unlike other viral and cellular activators that recognise specific sequence elements in promoter DNA, Tat recognises a hairpin loop structure, known as TAR, near the 5′ end of the viral RNA. And secondly, in contrast to all other activators, Tat does not have much effect on the frequency of RNA chain initiation. Rather, the main effect of Tat is to stimulate RNA polymerase II processivity, causing an increase in the fraction of transcripts that extend all the way to the 3′ end of the provirus. Without Tat, most viral transcripts terminate prematurely at many positions throughout the 9.5 kilobase proviral genome.

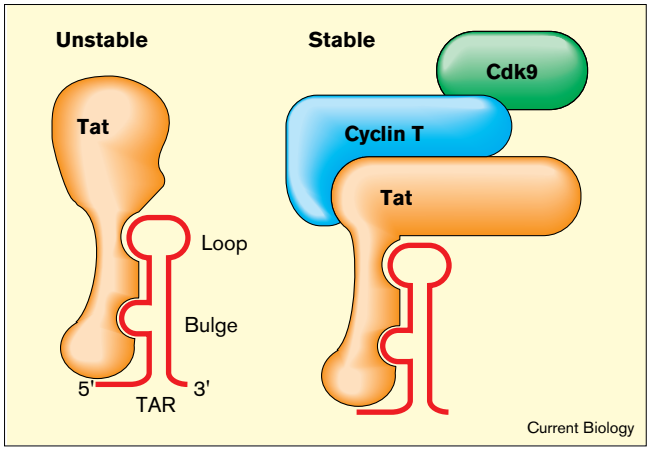
How can Tat, which binds to the 5′ proximal region of the RNA, stimulate elongation by polymerase II through distal arrest sites? Recently, important clues towards a solution of this puzzle have been discovered. These exciting findings draw together three previously disparate strands of evidence: a species-specific cofactor for Tat encoded by a gene that maps to human chromosome 12; a protein kinase that binds to Tat and phosphorylates the carboxy-terminal heptad-repeat domain (CTD) of the RNA polymerase II large subunit; and a ‘positive transcription elongation factor’ (P-TEFb) purified from *Drosophila* cells.

P-TEFb was identified on the basis of its ability to stimulate the production of long RNAs *in vitro* when added to polymerase II transcription complexes shortly after initiation [1]. This activity of P-TEFb is sensitive to the protein kinase inhibitor dichloro-ribofuranosyl benzimidazole (DRB), which selectively blocks synthesis of long RNAs, such as Tat-activated HIV-1 transcripts, without affecting the production of short RNAs such as those produced in the absence of Tat [2]. Price’s group sequenced two subunits of P-TEFb and found that they correspond to a previously discovered cyclin-dependent kinase, Cdk9, and a new partner, cyclin T [3,4]. The same cyclin-dependent kinase was identified independently as a kinase that bound *in vitro* to the activation domain of Tat and phosphorylated the RNA polymerase II CTD [5]. Cyclin T was also identified by Wei *et al.* [6] as a protein that bound to a Tat affinity resin.

The functional significance of Cdk9–cyclin T was demonstrated by its immunodepletion from transcription extracts, which was found to inhibit activation by Tat as well as basal transcription [3,6,7]. Other accessory proteins are associated with Cdk9–cyclin T, including Tat-SF1 (Q. Zhou, personal communication), a previously reported cellular cofactor for Tat [8]. The importance of P-TEFb was further supported by the results of a screen through 100,000 compounds for inhibitors of Tat function [7]. Among the compounds that emerged from this screen, a compelling correlation was found between their ability to inhibit Tat activity, on the one hand, and P-TEFb kinase activity, on the other.

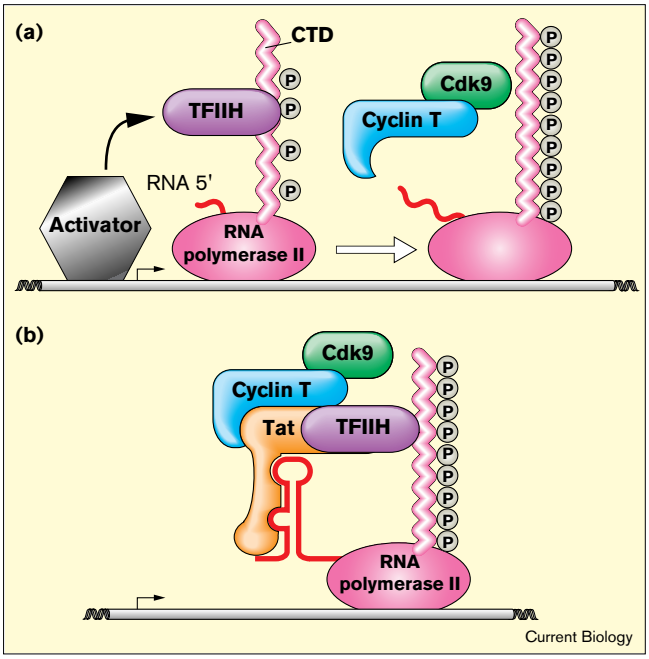
The interaction of P-TEFb with Tat has a fascinating effect on recognition of TAR. Pure Tat binds exclusively to residues in the bulge of the TAR hairpin structure, but residues in the loop are critical for activation of transcription by Tat. Elegant experiments by Wei *et al.* [6] have now provided a solution to this paradox. These workers discovered that cyclin T binds directly to Tat, that the cyclin T–Tat complex binds TAR with higher affinity than Tat alone, and

Figure 1



Cyclin T binds directly to the Tat activation domain, conferring tight binding to TAR RNA and recognition of the loop [6].

Figure 2



(a) A model for the two-stage phosphorylation of RNA polymerase CTD, first by TFIIH and then by Cdk9–cyclin T (P-TEFb). **(b)** Tat binding to P-TEFb and TFIIH may enhance the efficiency of CTD phosphorylation, launching highly processive polymerase II elongation complexes.

that cyclin T confers recognition of the loop as well as the bulge (Figure 1). HIV-1 appears to have evolved a cunning scheme in which it 'cooperates' with a host-cell factor, cyclin T, that simultaneously enhances Tat binding to TAR and recruits the P-TEFb kinase that stimulates the processivity of viral transcription. Moreover, cyclin T fulfills the criteria for the species-specific host-cell factor: the human cyclin T gene maps to chromosome 12 and its over-expression in rodent cells is sufficient to permit Tat activation [6].

P-TEFb is not the only protein kinase that binds to the Tat activation domain. The multi-subunit general transcription factor TFIIH, which has its own tripartite protein kinase, Cdk7–cyclin H–MAT1, binds to the same domain of Tat [9–12]. Like P-TEFb, TFIIH kinase phosphorylates the polymerase II CTD. Specific inhibition of Cdk7 by a pseudosubstrate peptide was found to reduce polymerase II processivity in the presence of Tat, suggesting that this kinase is required in addition to P-TEFb for Tat function [11]. TFIIH cannot substitute for P-TEFb in stimulating elongation *in vitro*, so these factors perform non-redundant functions [13].

RNA polymerase II is recruited to promoters in its hypophosphorylated IIA isoform, and then gets converted to the II0 form by multiple phosphorylations of the large subunit's CTD, comprising a heptad sequence (consensus YSPTSPS) which is repeated 52 times. The idea that polymerase II elongation is regulated by CTD phosphorylation has been popular for several years, and is substantially strengthened by the new evidence for interaction between Tat and CTD kinases. Both TFIIH and P-TEFb can convert polymerase IIA to the II0 isoform *in vitro*, and both are sensitive to the elongation inhibitor DRB, but there are also important differences between them.

Although Tat stimulates phosphorylation of the CTD by TFIIH [9–11], there is no evidence that it also enhances P-TEFb activity. Furthermore, TFIIH and P-TEFb act at two different times in the transcription reaction. TFIIH is a component of the pre-initiation complex that assembles on the promoter. It acts during initiation and promoter clearance, and is released when the RNA chain is 30–50 bases long [14]. In contrast, P-TEFb is not part of the pre-initiation complex. It works later in the reaction, during a window between when the polymerase clears the promoter — with a nascent RNA chain of about 15 bases — and when the transcript reaches about 500 bases [15].

These findings suggest a two-stage model for CTD phosphorylation, in which TFIIH acts first during the transition from initiation to elongation, and P-TEFb then acts as the transcript elongates through the first few hundred bases. The slow initial kinetics of CTD phosphorylation by P-TEFb *in vitro* [13] suggest that this kinase's activity might be enhanced if the substrate is first phosphorylated by TFIIH. Tat may be so effective at stimulating elongation because it integrates the two stages of CTD phosphorylation by contacting both TFIIH and P-TEFb (Figure 2). It remains to be determined whether the association of Tat with TFIIH and P-TEFb is sequential or simultaneous. In either case, it is possible that these two kinases communicate by phosphorylating one another, possibly under the influence of Tat (see Figure 2). The recent advances thus suggest a model in which Tat bound to TAR installs two kinases, which together make an efficient CTD phosphorylation machine that launches highly processive polymerase II complexes.

The breakthroughs in understanding how Tat works have important implications for the mechanism of activation by other viral and cellular transcription factors. Tat is not alone in its ability to stimulate polymerase II processivity, although it appears to be unique in so far as it almost exclusively affects this step in the transcription cycle. Other more conventional activators that stimulate elongation as well as initiation include herpes simplex virus VP16 and adenovirus E1a, and the cellular proteins p53 and E2F. All of these factors, like Tat, bind to TFIIH [12]. Whether P-TEFb interacts directly or indirectly with activators others than Tat will no doubt shortly be resolved, though it is obvious that P-TEFb function is not limited to HIV-1 transcription. P-TEFb was, after all, originally isolated as a

factor that stimulates the elongation of transcripts from *Drosophila* promoters. Moreover, Cdk9–cyclin T overexpression in mammalian cells stimulates Tat-independent gene expression from a cytomegalovirus promoter [4]. Exactly how P-TEFb is brought into play in the absence of Tat is still not known.

Despite the large body of evidence indicating that CTD phosphorylation positively affects the processivity of transcription, the underlying mechanism is completely unknown. CTD phosphorylation could work in various ways — it might influence the intrinsic catalytic properties of polymerase II, recruit elongation factors, or counteract inhibitors of elongation such as the recently discovered 'DRB sensitivity inducing factor' (DSIF) [16]. CTD dephosphorylation might also have an important regulatory function, and in this regard it is intriguing that a recently identified CTD phosphatase binds directly to the elongation factor TFIIF [17].

How does Tat bound to the 5′ end of HIV-1 RNA act at a distance to prevent premature termination of transcription throughout the provirus? It is possible that the pattern of CTD phosphorylation set up by Tat, TFIIH and P-TEFb at the beginning of a round of HIV-1 transcription is stable enough to maintain high processivity until the polymerase reaches the 3′ end of the provirus. Alternatively, it may be necessary to maintain the phosphorylation state of the elongating polymerase constantly, perhaps by hit-and-run binding of P-TEFb. In this regard, it is interesting to note that Tat associates with polymerase II complexes [18] and may actually travel as a passenger with the enzyme during transcript elongation [19].

CTD phosphorylation has been implicated not only in controlling elongation of nascent transcripts in response to activators, but also in co-transcriptional processing of those transcripts. The 5′ ends of eukaryotic pre-mRNAs are 'capped' with a structure that is important for their subsequent processing, transport and translation: this capping is dependent on the polymerase II CTD and involves recruitment of the capping enzymes specifically to the phosphorylated CTD [20]. It is also possible that association of splicing and polyadenylation factors with an 'mRNA factory' complex is influenced by CTD phosphorylation [21]. CTD phosphorylation under the direction of Tat and perhaps other transcription factors may therefore influence multiple post-initiation events in mRNA synthesis. High polymerase II processivity in response to CTD phosphorylation could be just one manifestation of a commitment by the gene expression machinery to synthesize a full-length and completely processed mRNA.

Acknowledgements

We thank David Price and Susan McCracken for helpful discussions. The authors' work is supported by a grant from the Medical Research Council of Canada.

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